An Assay Led Astray:

A curious case of biotin-induced hyperthyroidism, and disparity in biotin immunoassay interference.

Tashunka Taylor-Miller[1], Ashley Alexander[2], Gary Liu[3], Christina Trambas[3], Tina Yen[3], Michele O'Connell[1,4]
Departments of Endocrinology & Diabetes (1), General Medicine (2), Chemical Pathology (3), Murdoch Children's Research Institute (4) Royal Children's Hospital, Melbourne, AUSTRALIA , Poster Number: P3-P393

The Royal Children's Hospital Melbourne

Clinical Case Presentation

- Term, male , birth weight 3.24kg, not dysmorphic, nil liver edge.
- Family history 2 previous neonatal deaths (severe jaundice & seizures) day 3 of life.
- Concerns possible Inborn Error of Metabolism (IEM), started prophylactic phototherapy and oral pyridoxine, thiamine, biotin.
- General diagnostic work up; mildly elevated fT4 and fT3 (see Table 1A).
- Day 10 baby irritable with TFTs suggestive of significant biochemical hyperthyroidism. No clinical signs of hyperthyroidism; normal pulse rate, gaining weight, feeding well.
- Commenced Carbimazole (CBZ) 0.2mg/kg/dose.
- Day 10 sample was re-analysed on Abbott Architect (a platform not susceptible to biotin interference) and the baby was confirmed as euthyroid.
- Baby remained well without features of IEM thus biotin was ceased on day 10, and CBZ was ceased on day 12 (8 doses total administered).

Immunoassays

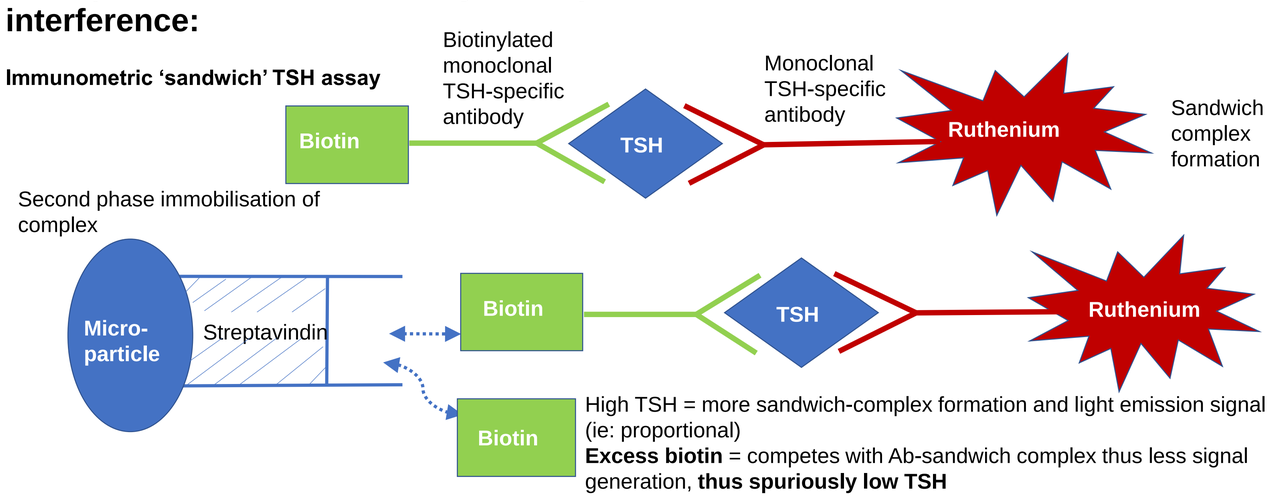
- Biotin affects variety of immunoassays: TFT, testosterone, oestrogen, FSH, cortisol, folate, ferritin.[1] Spurious biochemical hyperthyroidism due to biotin interference has been recently described in case reports.[2-5]
- On certain immunoassays (Table 1B) biotin can cause false and misleading TFTs.
- Retrospectively, samples from Day 4 & 5 were also re-analysed across multiple platforms and high levels of serum biotin were confirmed via Liquid chromatography–mass spectrometry (LCMS)
- When biotin reaches ≥ 30 ng/mL interference is apparent in biotin-prone immunoassays.
- Suggestion to withhold biotin for 72 hours before blood tests to minimise interference.

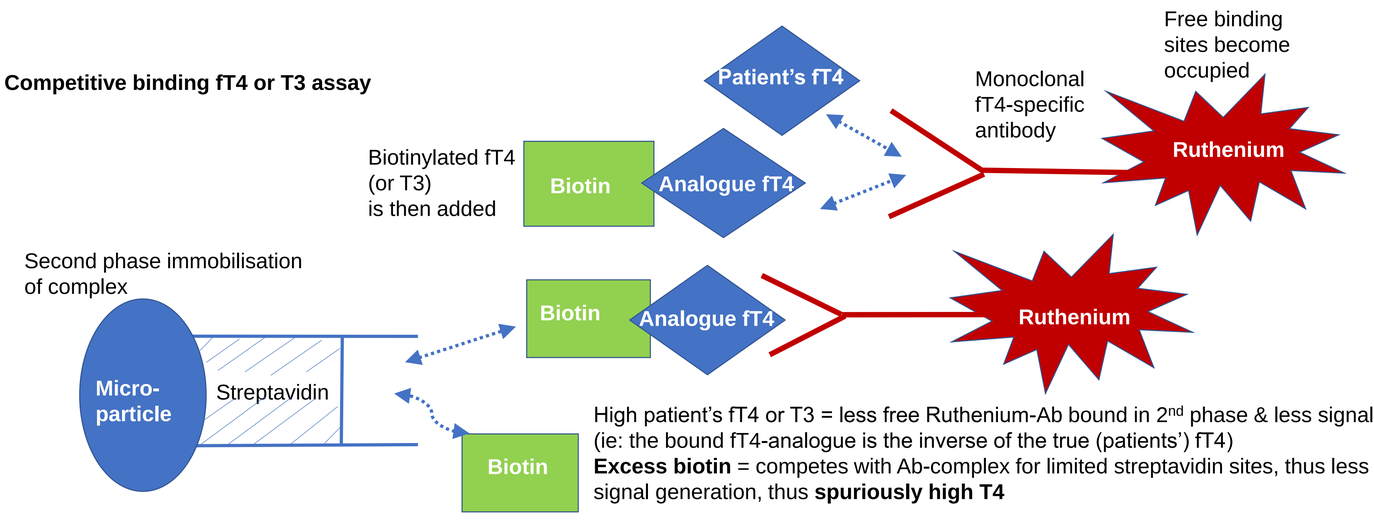
Table 1A. Impact of biotin on patient sample

Age of Infant	IMMUNOASSAY	VITROS	ROCHE	ARCHITECT	LC-MS Biotin∓
Day 2	TSH mIU/L	0.21[aV] (0.5-8.5)			
	fT4 pmol/L	44.5 (18-27)			
	fT3 pmol/L	9.3 (4.2 – 8.3)			
Day 4	TSH		<0.04[V] (0.43 – 16.10)		1008 ng/mL
	fT4		>100[^] (8.5 – 39.8)		
	fT3		15[^] (3.1-6.8)		
Day 5	TSH	0.02[cV]	0.04[V]		966 ng/mL
	fT4	36.4	>100[^]		
	fT3	7.6	14.3[^]		
Day 9	TSH		<0.05[bV]		
	fT4		100[^]		
	fT3		33.4[^]		
Day 10	TSH		0.09[V]	3.81[d] (0.88 – 5.42)	
CBZ started & Biotin ceased	fT4		>100[^]	19.4 (9.0 – 19.0)	
	fT3		19.3[^]		
Day 12	TSH		1.45		
CBZ ceased	fT4		32.1		
	fT3		9.2		
Day 16	TSH		7.05		
	fT4		19.9		
Day 23	TSH		6.81		
	fT4		17		

Key:
^ Value above reference range for age for the relevant platform
V Value before reference range for age for the relevant platform
∓ Biotin levels in these samples were confirmed through LC-MS analysis
Samples reanalysed retrospectively

Example: Roche Immunoassay susceptible to biotin interference:





Biotin (Vitamin B7)

- Water soluble vitamin, cofactor for carboxylases involved in fatty acid metabolism, leucine degradation, and gluconeogenesis.[1]
- RDI for children is 5-25 µg/kg/day.
- Therapeutic doses metabolic disorders 2-15 mg/kg/day.
- Many over-the-counter vitamin supplements contain biotin.

Table 1B. Impact of biotin on Immunoassay Profiles

	VITROS	ROCHE	ARCHITECT	BECKMAN	SIEMENS CENTAUR	SIEMENS VISTA	SIEMENS IMMULITE
TSH mIU/L	Affected - decreased	Affected - decreased	Unaffected	Unaffected	Unaffected	Affected - decreased	Unaffected
fT4 pmol/L	Unaffected	Affected - increased	Unaffected	Affected - increased	Unaffected	Affected - increased	Unaffected
fT3 pmol/L	Unaffected	Affected - increased	Unaffected	Affected - increased	Unaffected	Affected - increased	Unaffected

References:
1. Elston M, Sehgal S, Toit Du S, et al. Factitious Graves' Disease Due to Biotin Immunoassay Interference—A Case and Review of the Literature. The Journal of Clinical Endocrinology & Metabolism. 2016 Sep;101(9):3251–5.
2. Kwok J, Chan I, Chan M. Biotin interference on TSH and free thyroid hormone measurement. Pathology. 2012 Apr;44(3):278–80.
3. Wijeratne N, Doery J, Lu Z. Positive and negative interference in immunoassays following biotin ingestion: a pharmacokinetic study. Pathology. 2012;44(7):674–5.
4. Kummer S, Hermsen D, Distelmaier F. Biotin Treatment Mimicking Graves' Disease. N Engl J Med. 2016;375(7):704–6
5. Evans N, Yates J, Tobin J, McGill J, Huynh T. Immunoassay interference secondary to therapeutic high-dose biotin: A paediatric case report. JPCH. 2018;54:572-575

Take Home Points

- **Neonatal thyrotoxicosis is rare, particularly in the absence of maternal Graves' Disease.**
- **High-dose biotin only affects some TFT platforms. If affected, some or all of the constituent tests may be spurious and effects vary by analytical platform.**
- **Knowledge of biotin immunoassay interference is important for clinicians to correctly identify and/or respond to discordance laboratory results in high dose biotin treated infants.**

The Children's — Excellence in clinical care, research and education